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REVIEW OF LITERATURE ON HEALTH EFFECTS OF CORN OIL AND ITS OXIDATION PRODUCTS

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PREFACE

The work described in this report was authorized under Job Order No. 0WEE1D, Engineering Directorate, Protection Factor Testing Facility, U.S. Army Edgewood Chemical Biological Center. The work was started in April 2010 and completed in July 2010.

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REVIEW OF LITERATURE ON HEALTH EFFECTS OF CORN OIL AND ITS OXIDATION PRODUCTS

1. INTRODUCTION

This report summarizes information acquired from available literature on the health effects of corn oil (CO) and its oxidation products. The review specifically focused on CO and the oxidation products of CO produced under the environmental conditions in the exposure chamber operated by the U.S. Army Edgewood Chemical Biological Center (ECBC), Engineering Directorate, Protection Factor Testing Facility (Building E5604, Aberdeen Proving Ground, MD). These testing operations involved the generation and use of CO as an aerosol.

As part of representative testing, a challenge aerosol concentration of ~20 to 40 mg/m³, polydispersed CO aerosol having a mass median aerodynamic diameter (MMAD) of 0.4–0.6 µm (the Army Standard), is generated in a 10 × 10 × 32 ft test chamber, described in Campbell et al.¹ The test chamber challenge aerosol is generated by atomizing liquid CO at room temperature using an MSP Corporation Model 2045 High Output Aerosol Generator (MSP Corporation, Shoreview, MN). In preparation for tests, a chamber operator enters the test chamber that contains the challenge aerosol and ambient air, without wearing respiratory personal protective equipment (PPE). Upon completion of each test, a purge fan is run to evacuate the aerosol from the chamber. The chamber walls and windows are cleaned approximately twice a year with Windex™, dish soap, and water; mention of commercial products within this review does not constitute an endorsement of any commercial product. The maximum annual exposure by an operator to test chamber atmospheric constituents during test preparation is not expected to exceed 12 h/year, calculated as 15 min exposure on one day per work-week (based on testing that occurs one day per week, and 48 work-weeks/year).

2. REVIEW OF LITERATURE

CO is among several inward leakage test agents for PPE that have been proven effective by the National Institute for Occupational Safety and Health (NIOSH).² The NIOSH report detailed the following advantages of using CO for inward leakage testing:

- Easily attainable
- No risk to test subject
- No inherent dangers with storage or handling
- Well known
- Available instrumentation
- Established test methods

However, the report acknowledged that questions such as "Is CO appropriate for testing a gas mask?" have yet to be answered.²

The following are the main constituents of CO:

- Polyunsaturated fatty acids (PUFA), linoleic acid and a small amount of linolenic acid (together ~54%)
- Monounsaturated oleic acid (~25%)
- Saturated palmitic acid (~10%)
- Saturated stearic acid (<2%)
- Total triglyceride content (~95%)³

CO has been used extensively without incident as a vehicle to administer test chemicals by gavage (forced ingestion) in a variety of toxicity tests and dietary studies.⁴ The NIOSH 1983 report⁴ reviewed results of the studies available at that time, including those conducted by the National Cancer Institute (NCI). It revealed no mutagenic activity in *Salmonella* mutagenicity assays, negative findings for carcinogenicity in thousands of applications of refined CO to rodents in NCI studies, and occurrence of toxic responses to CO when its chemical composition was altered by heating to high temperatures (200 °C) or reused in frying foods. Therefore, the present review focuses on the results of the studies conducted since the publication of the NIOSH 1983 report⁴ except for the information on the inhalation toxicity of CO, which was acquired from all available sources, due to the special relevance of this exposure route for the purposes of this review.

2.1 Inhalation Effects

Available information on the inhalation toxicity of CO is limited to just a few studies. Inhalation of aerosolized oils, including vegetable oils, was reported to elicit a foreign body reaction that can result in exogenous lipid pneumonia (a rare form of pneumonia first described by Laughlin)⁵ and promote proliferative fibrosis in the lungs.^{6,7} Volk⁸ (cited in Banjar⁶) reported that lipid pneumonia monitored in 100 patients for up to 20 years revealed no lung cancer. However, a case of epidermoid carcinoma that developed in the middle lobe of a lung with extensive area of lipid pneumonia (in a 70 year-old female patient who used lightly medicated mineral oil-based nose drops) was reported by Keshishian et al.⁹ Four cases of oil aspiration pneumonia undergoing malignant degeneration, plus six similar cases cited from literature, were reported by Felson and Ralaisomay.¹⁰ The intratracheal injection of iodized (40% iodine) poppy seed oil, sesame oil, or olive oil caused no long-term damage to the lungs of test animals (puppies and rabbits) in the absence of infection.¹¹ With protracted exposure to lipid material, respiratory insufficiency may develop, occasionally leading to *cor pulmonale* (right ventricular enlargement secondary to a lung disorder that produces pulmonary artery hypertension).^{12,13} None of these conditions were specifically attributed to inhalation of aerosolized CO.

Shoskes et al.¹⁴ investigated the inhalation toxicity of refined CO using mice exposed to 12.6 g/m³ CO aerosol, with a mass median particle diameter ranging from 2.6 µm (40%) to 6.9 µm (14%). This exposure rate is 300 to 600 times greater than the CO aerosol challenge concentration being used in representative testing administered by the Protection Factor & Toxic Chamber Team. In the Shoskes et al.¹⁴ investigations, examination of lungs after a 2 h exposure or longer exposures of 4 to 8 h/day (5 days a week for one month) revealed that

oil droplets were immediately and actively phagocytosed by macrophages, resulting in nearly complete clearing of lungs from oil droplets within 48 h, with no inflammatory changes. Inhalation of refined CO did not irritate the upper respiratory tract and did not induce reflex apnea or bronchoconstriction.¹⁴

2.2 Reproductive and Developmental Effects

CO has generally been assumed to be biologically inert with regard to reproductive performance and developmental status,¹⁵ using the gavage exposure route (i.e., not inhalation). However, the results of several studies suggested the need to examine with caution such assumptions regarding ingestion and gavage exposures. Schmidt and Abbott¹⁶ reported that mitogenic reactivity of the T-lymphocyte compartment of rat offspring's spleen was significantly impaired by prenatal treatment of Long-Evans rats with CO (0.5 mL by gavage). They hypothesized that the maternal administration of CO can preferentially stimulate lymphocyte proliferation within the developing spleen.

In a follow-on study with C57BL/6J mice, CO was further implicated as a developmental toxicant that affects postnatal murine immune function following prenatal administration (250 μ L by gavage).¹⁷ A feeding diet containing 20% CO for Sprague-Dawley rats during pregnancy and lactation resulted in significant increases in the activity of hepatic microsomal oxidative enzymes in the male offspring at weaning, puberty, and adult stage of life. The female offspring had similar but less marked effects.¹⁸ These authors suggested that in progeny, such changes can alter the metabolism, toxicity, and pharmacological action of certain drugs and environmental pollutants. The combination of CO (10 mL/kg) with a diet containing animal protein, administered to female Sprague-Dawley rats, significantly reduced body weight gain on days 0–4 of lactation and viability of pups, and resulted in necrosis and fatty degeneration of the kidneys of dams.¹⁹ The same CO dose combined with a diet containing plant-based protein had no adverse effects on the rats used in that study.

In another study, a single 0.2 mL intramuscular injection-dose of corn oil into female Wistar rats resulted in the following significant changes (compared with controls):

- Delay in fertilization
- Decrease in fertility index
- Decrease in number of *corpora lutea* per dam (*corpus luteum* - a yellow, progesterone-secreting mass of cells that forms from an ovarian follicle after the release of a mature egg)
- Decrease in implantation sites per litter
- Decrease in living fetus in the uterus, thereby increasing the number of dead fetuses.²⁰

In a retrospective comparison of historical teratology study data for vehicle control animals (Sprague-Dawley derived albino rats [CD] and Swiss albino mice [CD-1]) treated by gavage with distilled water (DW) or CO during organogenesis, Kimmel et al.²¹ established that the percent fetuses malformed per litter and the average number of defects (malformations and variations) per fetus per litter were significantly higher in CO- than in

DW-treated litters for both rodent species. A consistent pattern of increased malformation incidence in CO-treated litters was observed across a 4 year period (1980–1983) in both species.²¹ However, Price et al.¹⁵ remarked that differences between CO and DW groups could not be conclusively attributed to the effect of treatment because historical control data had been collected over a 4 year period and therefore did not provide carefully controlled conditions under which these vehicles could be coherently compared.

In the controlled comparison study, using groups treated concurrently with CO and DW vehicles (5 mL CO or DW/kg by gavage), Price et al.¹⁵ failed to find treatment-related differences on those maternal endpoints, which had exhibited statistical significance in the retrospective non-coherent comparison. In regard to indices of embryo/fetal development, the coherent study by Price et al.¹⁵ found no statistically significant differences between groups for average fetal body weight (gestational day [gd] 20) or for any of their measurement endpoints for prenatal viability. Likewise, no significant difference was observed for the percentage of malformed fetuses per litter, which was slightly higher in the CO group due to the occurrence of one litter containing 100% (1/1) malformed fetuses. Other indices of malformation incidence in the CO group were actually lower than, but not significantly different from, the DW group.¹⁵ Similar coherent findings and conclusions for CO have been reported in a comparison of CO and DW (3 or 10 mL/kg, each) following administration by gavage to CD-1 mice on gd 6–15,²² no effect of treatment on any measure of developmental toxicity, except for a marginal but non-significant increase in the incidence of one anatomical variation (supernumerary rib) in the high dose (10 mL/kg CO) group.²²

2.3 Tumorigenesis

Diets containing CO (i.e., exposure routes other than inhalation) have been shown to enhance carcinogenesis in many organs. The 2 year toxicology and carcinogenicity studies by the National Toxicology Program²³ demonstrated that CO (as well as safflower oil or tricaprylin, also used in the studies) administered by gavage at a volume of 2.5, 5, or 10 mL/kg body weight once daily, 5 days/week, was not toxic. However, it caused significant dose-related increase in incidences of hyperplasia and adenoma of the exocrine pancreas in male F344/N rats.

Diet containing 23.5% CO promoted colon carcinogenesis in male F344 rats by up-regulating the cyclooxygenase-2 (COX-2) expression.²⁴ Lung carcinogenesis induced by 4-nitroquinoline 1-oxide (4NQO) in mice was promoted by a 20% CO-containing diet.²⁵ Lung carcinogenesis induced by 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in F344 rats was promoted by a 23.5% CO-containing diet (NNK-high CO-diet treatment) for 18 months, with no significant difference compared with a NNK-low (5%) CO diet treatment at the end of the 24 month study.²⁶ In the same study, Hoffmann et al.²⁶ demonstrated that an NNK and high CO diet enhanced and accelerated pancreas tumorigenesis.

Feeding male F344 rats a diet containing 10% CO, following the individual administration of different carcinogenic chemicals, increased the incidence and multiplicity of adenomas, carcinomas, total colon tumors, and a tendency to lung tumor formation. The diet significantly decreased the numbers and/or areas of glutathione S-transferase placental (GST-P) positive foci and putative preneoplastic lesions of the liver compared to the control group-fed

basal diet containing 5.1% CO in cake mixture.²⁷ Treatment with CO did not affect the incidences of neoplastic or preneoplastic lesions in the thyroid, tongue, esophagus, forestomach, small intestine, or urinary bladder, as compared to the control group.²⁷

Administering CO to the B6C3F1 male mice (0.15 mL/day, 5 days/week via gavage) injected with diethylnitrosamine (DENA, 5 µg/g bw) significantly decreased the number of DENA-induced hepatic adenomas compared with DENA only treated mice.²⁸ Administering a high fat-content diet containing 8 or 40% CO to young adult Sprague-Dawley female rats enhanced the development of DMBA [7,12-dimethylbenz(a)anthracene]-induced mammary cancers, especially the number of tumors per animals, without any endocrinologic and caloric alterations in the cancer bearers.²⁹

In a review of the studies designed to examine the relationship between dietary fat and calories in experimental rodent mammary gland tumorigenesis, Welsch³⁰ concluded that hyperalimentation of fat, either saturated (such as lard and beef tallow) or unsaturated (derived from vegetable products, e.g., CO, sunflower seed oil, safflower seed oil) significantly stimulated this tumorigenic process, as had been demonstrated in an array of carcinogen-induced, transplantable, spontaneous, and metastatic experimental rodent mammary gland tumor systems.

2.4 Oxidation of CO

Few studies have investigated oil oxidation at room temperature.^{31,32} During autooxidation of PUFAs of edible oils, including CO, the triglyceride structure undergoes changes, which lead to the formation of primary and secondary oxidation compounds, such as fatty acid hydroperoxides and conjugated-dienic systems. These include alkanals, (*E*)-2-alkenals, (*E,E*)-2,4-alkadienals, and γ -oxygenated α,β -unsaturated aldehydes with demonstrated genotoxicity and cytotoxicity.^{31,33} Studies by Guillén and Goicoechea³¹ revealed that CO samples stored at room temperature (20–25 °C) in closed containers for 12–121 months exhibited a preferential degradation of linoleic acyl groups.

No intermediate oxidation compounds were detected in CO samples stored for 12 months. Samples with low levels of oxidation (18–74 months storage time of CO samples with variable air-oil contact surface, air or oil volumes, and air-oil volume ratios) contained from 0.21 to 0.3 relative molar proportions (rmp) of hydroperoxides, with small proportions (0.05–0.19 rmp) of which (*Z,E* isomers) had conjugated double bonds. Samples with intermediate oxidation levels (from 99 months storage time onwards) contained, in addition to the above mentioned hydroperoxides, hydroxy derivatives having (*Z,E* isomers) conjugated-dienic systems. Samples with advanced oxidation state contained hydroperoxides having (*E,E* isomers) conjugated double bonds and aldehydes. Among aldehydes, the main constituents were alkanals, (*E*)-2-alkenals, and 4-hydroxy-(*E*)-2-alkenals; in addition, there were (*E,E*)-2,4-alkadienals, 4-hydroperoxy-(*E*)-2-alkenals, and in very small proportions 4,5-epoxy-(*E*)-2-alkenals. Among the aldehydes detected by Guillén and Goicoechea,³¹ the γ -oxygenated α,β -unsaturated are very reactive, whether they constitute molecules of low molecular weight, or are bonded to truncated triglycerides.

Among the latter molecules, the best-known is 4-hydroxy-(*E*)-2-nonenal (HNE), which is highly toxic to mammalian cells,³⁴ and whose biological activity has been a subject of great concern.³³⁻³⁶ Small amounts of acrolein, which is highly cytotoxic towards mammalian cells, can also be formed from PUFAs during lipid peroxidation.³³ When inhaled, acrolein is a highly selective respiratory tract toxicant, inducing a cell death pathway in human bronchial epithelial cells.³⁷ Based on a comprehensive literature review by Esterbauer et al.,³³ HNE treatments of different cell lines at concentrations ranging from 10–200 μ M produced the cytotoxic effects that were cell type-dependent. Doses of \sim 100 μ M HNE (concentration of any aldehyde that is unlikely to be attained in cells or organs outside of experimental conditions) were lethal, and killed all the cells in <90 min in Human umbilical vein endothelial cells, fibroblasts, and Chinese hamster ovary cells; whereas Ehrlich ascites tumor cells (EATC) or isolated hepatocytes were much more resistant and not lethally damaged by the same dose of HNE. Concentrations of HNE in the range of 1–20 μ M inhibited DNA and protein synthesis, stimulated phospholipase A 2, and inhibited c-myc expression. Effects produced by 0.1 μ M HNE or less (a basal physiological level in many tissues, as well as in serum) included stimulation of oriented migration of rat neutrophils, (i.e., chemotaxis), modulation of adenylate cyclase activity, weak stimulation of guanylate-cyclase, and stimulation of phospholipase C. Overall, the effective doses for HNE were in the range from 10^{-6} to 10^{-9} M in studies reviewed by Esterbauer et al.³³

The more recent studies have shown that other γ -oxygenated α,β -unsaturated aldehydes detected in the study by Guillén and Goicoechea,³¹ such as 4,5-epoxy-(*E*)-2-decenal, have reactivity that is similar to HNE.³⁸ Jian et al.³⁸ suggested that these lipid hydroperoxide-derived bifunctional electrophiles may play an important role in cardiovascular pathology through their ability to induce endothelial cell apoptosis. Likewise, it has been suggested that truncated 4-hydroxy-(*E*)-2-alkenal phospholipids may rapidly react with proteins forming covalent adducts and modulate many of the cellular events, including the formation of foam cells, which are precursors of the atherosclerotic plaques, among other biological activities.³⁹⁻⁴³ The discovery by Guillén and Goicoechea³¹ of these toxic compounds in CO samples stored at room temperature may explain the results obtained by Perjesi et al.,⁴⁴ who treated CBA/Ca inbred mice with CO samples stored for 48 months, either at room temperature or in the refrigerator, and found significantly increased expression of the Ha-ras gene in all the investigated organs (liver, lung, kidney, thymus, and spleen) of the rancid CO-treated animals. In that study,⁴⁴ after the rancid CO treatment, expressions of the c-myc and the p53 genes were also increased in all the organs except the thymus of the mice. Based on these results, the authors concluded that rancid oils, rich in omega-6 unsaturated fatty acids, could be involved not only in tumor promotion, but in initiation as well.⁴⁴

3. CONCLUSIONS

This report reviews available data on the health effects of corn oil (CO) and its autooxidation products, which were exposed to the environmental conditions in the exposure chamber used by the Engineering Directorate's Protection Factor Testing Facility in Building E5604. Information on inhalation toxicity was of primary interest for this review due to the special relevance of this exposure route for a chamber operator. The health effects data for other

exposure routes (e.g., ingestion via gavage or free feeding in dietary studies, or intratracheal or intramuscular injections in toxicity tests) were included in this review only on the basis of providing a more comprehensive compilation of the information available on the overall health effects of CO.

Very limited information was available on the inhalation toxicity of CO. Although inhalation of aerosolized oils (including vegetable oils) was reported to elicit lipoid pneumonia, promote proliferative fibrosis in the lung, result in the development of respiratory insufficiency that may lead to *cor pulmonale* or educe carcinomas, none of these conditions were specifically attributed to inhalation of aerosolized CO. Investigation of the inhalation-toxicity of refined CO aerosol using mice exposure rates that were 300–600 times greater compared to a challenge CO aerosol concentration used in a representative test administered by the Protection Factor & Toxic Chamber Team, revealed that oil droplets were immediately and actively phagocytosed by macrophages. This resulted in nearly complete clearing of the lungs from oil droplets within 48 h with no inflammatory changes. Furthermore, CO is amongst several inward leakage test agents for personal protective equipment that have been proven effective by the National Institute for Occupational Safety and Health (NIOSH). NIOSH² reports that CO is easily attainable, does not put the test subject at risk, does not have inherent dangers with storage or handling, is well known, and has established test methods.

CO has been used extensively as a vehicle to administer test chemicals by gavage in toxicity tests and dietary studies (i.e., exposure routes other than inhalation). CO via these exposure routes has generally been perceived as biologically inert with regard to mammalian reproductive performance and developmental status. Studies researching exposure routes other than inhalation that did implicate CO as a reproductive or developmental toxicant, typically used considerably greater exposure concentrations than those that may be encountered by a test chamber operator, or relied on historical control data collected over several years. These features diminished the coherence and usefulness of these studies for comparison purposes as control treatments. Later studies, using the appropriate concurrent coherent controls, thus imparting greater confidence in the study results, failed to find any treatment-related differences between CO and distilled water vehicles (for the same assessment endpoints that had earlier been attributed statistical significance in the non-coherent retrospective comparisons).

In contrast to the effects on reproductive or developmental performance, studies that used ingestion or gavage exposure routes (as opposed to the inhalation exposure route) for experimental diets containing high quantities (8% or greater) of CO in diet have been shown to enhance carcinogenesis in many organs. Examples included dose-dependent increase in incidences of hyperplasia and adenoma of the pancreas, and colon carcinogenesis in male rats. Hyperalimentation of unsaturated fats, including CO, significantly stimulated mammary gland tumorigenesis, as had been demonstrated in an array of carcinogen-induced, transplantable, spontaneous, and metastatic experimental rodent mammary gland tumor systems. High CO diets also enhanced or accelerated carcinogen-induced tumorigenesis in the lungs and pancreas, and increased the incidence and multiplicity of adenomas, carcinomas, and total colon tumors. Treatment with CO by ingestion or gavage exposure routes did not affect the incidences of neoplastic or preneoplastic lesions in the thyroid, tongue, esophagus, forestomach, small intestine, or urinary bladder as compared to the control group. The relevance of these findings

for a chamber operator's exposure risk is uncertain, and may require further analysis especially if alteration of chamber operations in the future make these applicable work-related routes of exposure within the chamber operator's official duties.

Autooxidation of CO at room temperature has led to the production of harmful compounds during long-term (years) storage. However, the findings of a time-dependent nature of the formation of such oxidation compounds may make autooxidation of CO in short-term (months) storage inconsequential for a chamber operator's exposure risk from CO. The Protection Factor & Toxic Chamber Team purchases fresh CO in 1 gal containers (jugs) and disposes of any unused portions after the expiration date (typically <12 months; recommended shelf life of CO is 12–18 months). The unused portion of CO delivered into the aerosol generators is emptied every month; aerosol generators are re-filled with fresh CO. These and other practices employed by the Protection Factor & Toxic Chamber Team (i.e., purging the aerosol from the chamber upon completion of each test, periodically cleaning the chamber to remove oil residues from walls and windows, and limiting the time spent by an operator within the test chamber) further minimize the chamber operator's incidence of risk from exposure to CO constituents in the air within the test chamber.

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